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Effects of pH and Temperature on Reaction Kinetics of Catechins in Green Tea Infusion†

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Stability of tea catechins during heat processing was examined. Effects of pH and temperature on reaction kinetics of degradation of tea catechins were investigated. Reaction of −EC was accelerated at pH higher than 6.0, inhibited at those lower than 5.0. A dominant reaction of −EC in slightly acidic media was isomerization. The apparent first order reaction proceeded in slightly acidic media under sterilization conditions. A similar reaction proceeded for −EC, −EGC, −ECg, and −EGCg in green tea infusion at temperatures below 95°C. A turning point temperature on an Arrhenius plot was observed at 82°C. A great difference of apparent activation energies was observed lower and higher than 82°C. The reaction rate constant of catechins in slightly acidified tea infusion was less than half as much as the constant in the original infusion. The stability of catechins in green tea infusion was subject to the turning point temperature.

Canned or PET bottled drinks of black tea, oolong tea, and green tea are supplied as packaged beverages for consumer use. Most packaged tea drinks are low acid beverages, and are kept warm in the vending system during the cold season. High temperature heat processing for a fixed time is required to sterilize the spores of thermophilic anaerobes according to Japanese food hygienic regulations. Therefore, it is important to minimize changes of functional constituents in tea drinks during production, distribution, and storage.

Previous research[1,2] on the stability of constituents in canned or PET bottled tea drinks has shown that caffeine is highly stable but catechins are rather unstable and that the dominant change of catechins seems to be isomerization influenced by pH of the infusion at the heating. Recently, tea catechins received attention for their important tertiary functionalities, *e.g.*, antioxidative,[3] angiotensin-converting enzyme inhibiting,[4] antibacterial to foodborn pathogenic[5] and cariogenic[6] bacteria, antitumor,[7] anti-virus,[8] and amylase inhibition[9] activities.

The objective of our study is to establish an appropriate procedure of processing for the production of quality-stable canned or bottled tea drinks by obtaining information on thermal reaction kinetics of catechins in tea infusions. In this paper the effects of pH of infusion and heating temperature on the degradation reaction rate and apparent activation energies of catechins in green tea infusion are elucidated.

Materials and Methods

Catechins. Standard reagents of −EC (99.64% purity), −ECg (98.43%), +C (100.0%), −EGC (99.59%), and −EGCg (99.64%) were obtained from Kurita Kogyo Co., Ltd., Japan. Standard reagent of ±C was obtained from Extrasynthese Co., France.

Experimental apparatus. A reaction apparatus was designed to simulate the heating history of packaged tea drinks such as extraction, hot filling, and sterilization. A pressure-resistant hermetical glass tube (3.5 mm i.d.) fitted with a glass bead to shake the contents and a thermo-couple to monitor temperature of the solution was used. Three hundred μl of test solution was transferred to the tube by a syringe and the tube was sealed with a gas-tight stopper under nitrogen gas flushing to the headspace of the filled tube. The tube was dipped in an oil bath maintained at a constant temperature, shaken at a regular intervals, then cooled in a cold water flow. In the apparatus, the solution was heated up to 121°C within 30 seconds and cooled to ambient temperature within 5 seconds.

Analysis of catechins. Catechins were determined by the HPLC method described by Terada *et al.*[10] with a few modifications. The HPLC system (Shimadzu LC-6A) fitted with a UV detector (measured at 280 nm) and an ODS column (Ultron N-C18, 150 mm × 4.6 mm i.d.) was used. The temperature of the column was maintained at 43°C. Analysis was done by a gradient system. Phosphoric acid (0.1 v/v%) containing acetonitrile (0.1 v/v%) and *N*,*N*-dimethylformamide (5 v/v%) as A solution, and acetonitrile as B solution were used as the mobile phase with a flow rate of 1 ml/min. A sample solution was diluted with the same volume of acetonitrile, filtered by a membrane filter (0.45-μm pore size); 10 μl of the solution was injected. Catechins were identified by comparison of their retention time with authentic standards and determined by peak areas from the chromatograms.

Measurement of absorbance and pH. Contents of 10 tubes for each set of experimental conditions were transferred into a vessel by a syringe. Absorbance at 430 nm was measured by a spectrophotometer (Shimadzu UV-160A) and the pH of the solution was measured by a pH meter (Horiba M-13) in Experiment 1.

Preparation of (−)-epicatechin solution.

Experiment 1: Standard reagent of −EC was dissolved at a concentration of 8 mg/100 ml in deaerated, deionized water. Deaeration was done by nitrogen gas (99.99%) bubbling after evacuating and boiling of deionized water. Ten-ml portions of the solution were transferred to glass tubes. To adjust the pH of the solution in a range from 3.0 to 7.1, four kinds of acidic and basic solutions, 1% aqueous solution of citric acid, sodium citrate, AsA, and sodium hydrogen carbonate, were prepared. Each acidic or basic solution was added dropwise from a microsyringe to the −EC solution. The addition was designed by a preliminary test to obtain a target pH.

Experiment 2: Standard reagent of −EC was dissolved at a concentration of 8 mg/100 ml in deaerated McIlvaine buffer solution (a mixture of 0.1 M

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Abbreviations: −EC, (−)-epicatechin; −ECg, (−)-epicatechin gallate; −EGC, (−)-epigallocatechin; −EGCg, (−)-epigallocatechin gallate; +C, (+)-catechin; ±C, (±)-catechin; −C, (−)-catechin; AsA, L-ascorbic acid; PET, polyethylene terephthalate.

citric acid and 0.2 M disodium hydrogen phosphate). The pH of each medium was previously adjusted at 4.0, 5.0, 6.0, and 7.0 by changing the ratio of acidic and basic solution mixed.

Preparation of green tea infusion.

Experiment 3: Dried green tea leaves ('Yabukita') of a weight of 1% for a weight of treated water were extracted. Water treatment was done by ion-exchanging, UV-sterilization, activated charcoal treatment, and boiling of conventional city water. Extraction was done for 3 min at 60°C, the infusion was filtered with 250 mesh nylon cloth, then rapidly cooled to 30°C.

Experiment 4: To a part of the infusion, AsA (Wako Pure Chemical Industries, Ltd., Japan) was added at a concentration of 20 mg/100 ml.

The solution and the infusion thus prepared were used to measure degradation of −EC and the degradation reaction kinetics of catechins.

Results and Discussion

Experiment 1. Reaction of −EC in relation to the pH of the solution

Effects of pH and reductivity of solution on the reaction of −EC after the heat processing at 121°C for 4.4 min are shown in Fig. 1. The degradation of −EC was obviously dependent upon the pH of the solution. The reaction scarcely proceeded in slightly acidic media at pH below 5.0; however, it was accelerated at pH above 6.0. Concerning the reaction products, a peak corresponding to the retention time of +C or ±C appeared on the chromatogram after the heat processing in our study. −EC isomerizes to its corresponding epimer or racemic compounds by thermal treatment exceeding 100°C.[11,12] Courbat[13] reported that a rapid epimerization of catechins occurred in alkaline solution, and Nakagawa[14] pointed out that the isomerization of catechins in the roasting process of green tea was not racemization but epimerization due to the stereochemical configuration of 3-hydroxyfuravanon.

Therefore, it seemed that −EC changed to its epimer, −C. As +C and ±C had the same retention time and same molar absorbance on the HPLC chromatogram, the reaction product of −EC was determined as ±C in this study. The result showed that the reaction product proportionally increased with the decrease of −EC at pH below 5.5, greatly decreased at pH above 6.0, and browning of the solution was accelerated. These changes were commonly observed in media containing AsA as well as those containing citrate. Therefore, it seemed that the dominant reaction of −EC in slightly acidic media was not oxidation but isomerization and the degradation of the resultant product proceeded simultaneously in neutral media.

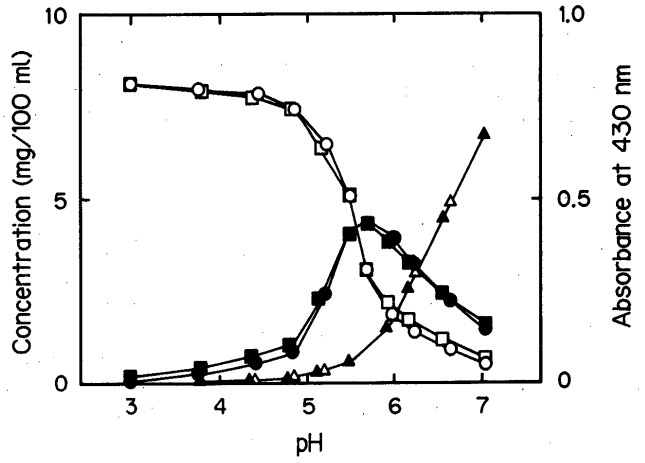

Fig. 1. Effects of pH on Reaction of −EC and Change of Absorbance at 430 nm in Citrate and Ascorbate Solution Heat-processed at 121°C for 4.4 min

Heating time is shown as actual holding time at 121°C. It was set to reach a sterilization value, F_0, of 4.0. Symbols: ○, retention of −EC in citrate solution; □, retention of −EC in ascorbate solution; ●, increase and decrease of reaction product, ±C, in citrate solution; ■, increase and decrease of reaction product, ±C, in ascorbate solution; △, absorbance at 430 nm of citrate solution; ▲, absorbance at 430 nm of ascorbate solution.

Experiment 2. Reaction kinetics of −EC in citrate-phosphate buffer

Citrate–phosphate buffer solutions adjusted to pH 4.0, 5.0, 6.0, and 7.0, containing 8 mg/100 ml of −EC, was heat-processed at 121°C for various times from 1 to 15 min. The concentrations of −EC in each buffer solution was determined immediately after the heat processing and the log of the ratio of the remaining −EC to the initial concentration was plotted for each pH–temperature processing against the heat processing time. An apparent first order reaction proceeded in slightly acidic media less than pH 5.0, but a different mode of reaction was observed in media exceeding pH 6.0 (Fig. 2). The result suggested that other reactions such as oxidation and/or polymerization[15] might simultaneously proceed under high-temperature processing in media exceeding pH 6.0. A decrease of −EC and an increase of ±C during processing were also observed. Here, the sum of −EC and ±C measured was almost equivalent to the initial −EC added in acidic media or at early processing time, *e.g.*, 99.1% for 15 min in the medium of pH 4.0 and 100% for 6 min in the medium of pH 5.0. Increased reaction product, ±C, finally decreased in neutral media with prolonged heat processing. These results were similar to that obtained in experiment 1. Therefore, it seemed that a dominant reaction of −EC in slightly acidic media was isomerization as far as the reaction fitted an apparent first order kinetics, and once other reactions such as oxidation and/or polymerization proceeded, the first order kinetics was disturbed.

In green tea infusions, degradation kinetics of catechins during the heat processing was examined at the temperature range in which an apparent first order kinetics was established. The behavior of resultant products was not discussed in this study due to difficulties of qualification and complexity of the kinetics.

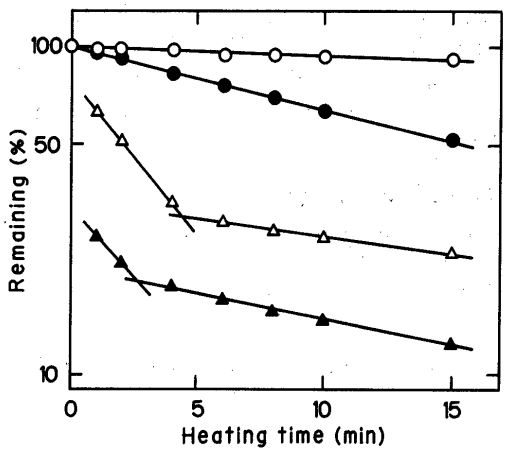

Fig. 2. Apparent First Order Reaction Rate Plot of −EC in McIlvaine Buffer Solution at Various pH under Sterilization Conditions at 121°C.

Heating time was shown as actual holding time at 121°C. Symbols: ○, pH 4.0; ●, pH 5.0; △, pH 6.0; ▲, pH 7.0.

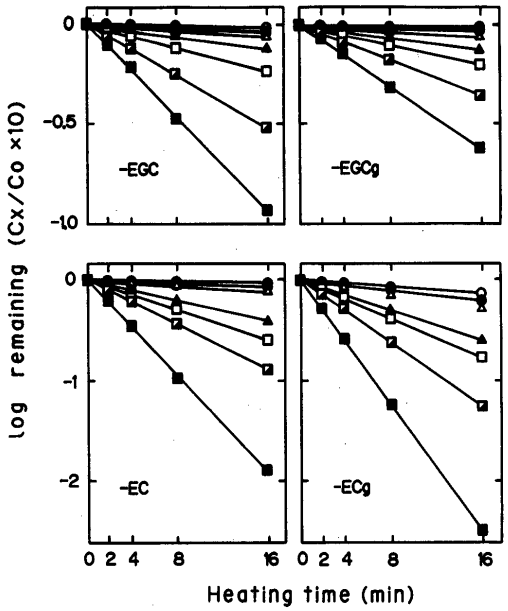

Fig. 3. Apparent First Order Reaction Rate Plot of −EGC, −EGCg, −EC, and −ECg in Green Tea Infusion at 25, 40, 55, 80, 85, 90, and 95°C.

C_o, initial concentration of −EGC, −EGCg, −EC, and −ECg before heat processing; C_x, concentration of −EGC, −EGCg, −EC, and −ECg immediately after heat processing. Initial concentration of catechins in green tea infusion: −EGC, 38.0 mg/100 ml; −EGCg, 31.6 mg/100 ml; −EC, 15.7 mg/100 ml; −ECg, 8.6 mg/100 ml. Symbols: ○, at 25°C; ●, at 40°C; △, at 55°C; ▲, at 80°C; □, at 85°C; ◪, at 90°C; ■, at 95°C.

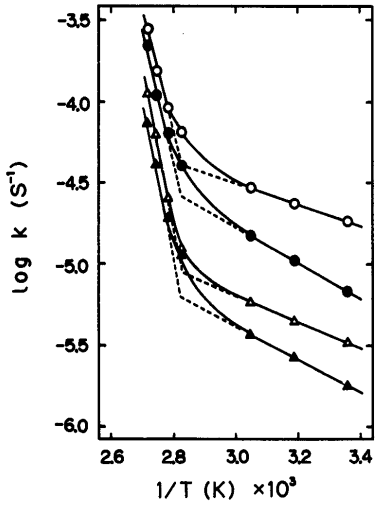

Fig. 4. Arrhenius Plot of Apparent First Order Reaction Rate Constants of −ECg, −EC, −EGC, and −EGCg in Green Tea Infusion.

k, apparent first order reaction rate constants of −ECg, −EC, −EGC, and −EGCg in green tea infusion. T, degrees absolute Kelvin. Symbols: ○, −ECg; ●, −EC; △, −EGC; ▲, −EGCg.

Experiment 3. Reaction kinetics of catechins in green tea infusion

The log of the ratio remaining to the initial concentration of −EGC, −EGCg, −EC, and −ECg in green tea infusions for each temperature was plotted against heating time (Fig. 3). The reaction fitted apparent first order kinetics for the four kinds of catechins at the temperature examined, but the reaction rate constants calculated from the slope of the best fit line were different for each kind of catechins.

The log of reaction rate constants k (s^{-1}) for each temperature processing in the range of 25°C to 95°C was plotted against the inverse of the absolute temperature. Each Arrhenius plot showed not a straight line but a concave one consisting of two straight lines which crossed each other at a specific turning point. The turning point was commonly observed on each Arrhenius plot of −ECg, −EC, −EGC, and −EGCg at 2.82×10^{-3} (1/K); at 82°C (Fig. 4). The turning point temperature on the Arrhenius plot suggested that there might exist two competing reactions of the same reaction products and a different temperature dependence, or two or more reactions of different reaction products and different temperature dependence.

Table Apparent Activation Energies of Degradation of −ECg, −EC, −EGC, and −EGCg in Green Tea Infusions

Catechins	Activation energies (kcal/mol)		
	E_{a1}(<82°C)	E_{a2}(>82°C)	E_{a2}/E_{a1}
−ECg	3.2	35.8	11.2
−EC	5.2	38.1	7.3
−EGC	3.6	41.1	11.4
−EGCg	4.7	37.9	8.1

E_{a1}, activation energy at lower than 82°C; 2.82×10^{-3} (1/K).
E_{a2}, activation energy at higher than 82°C; 2.82×10^{-3} (1/K).

The apparent activation energy was calculated at higher or lower temperatures than 82°C by multiplying the slope of the best fit line by the gas constant (Table). The apparent activation energies obtained were slightly different between the four kinds of catechins, but in comparison between the two values; E_{a1} and E_{a2}, E_{a2} was 7.3 to 11.4 times larger than E_{a1} for all catechins analyzed. This meant that the actual reaction rate measured at higher than 82°C was faster than the reaction rate predicted by extrapolation from the one measured below 82°C. In this study, the isomerization reaction of catechins proceeded in green tea infusions under relatively mild heating conditions below 95°C, and at lower or higher than the turning point temperature there might exist different modes of reaction. This suggested that a different temperature–time relationship existed in the thermal behavior of tea catechins beyond the boundary temperature; 82°C. Furthermore, this suggested that a careful consideration might be required at the hot-water extraction of green tea leaves to determine catechins concentrations.

Experiment 4. Effects of pH on reaction kinetics of tea catechins

The effects of pH on reaction kinetics of tea catechins were examined. A similar experiment was done with or without an addition of AsA to the green tea infusion. The resultant pH was 4.93 after the addition, while the pH of the original infusion was 6.12. The reaction also fitted an apparent first order kinetics in the AsA-added infusion (Fig. 5). Here, 'total catechins' were shown as the sum of −EC, −ECg, −EGC, and −EGCg to simplify the comparison.

The apparent reaction rate constant of catechins in the AsA-added infusion was less than half that in the original infusion. Concerning the apparent activation energies, E_{a1} in the AsA-added infusion was 2.5 kcal/mol and in the

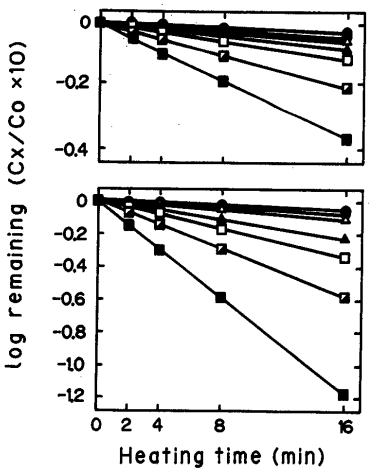

Fig. 5. Apparent First Order Reaction Rate Plot of 'Total Catechins' in Green Tea Infusions with or without an Addition of AsA at 25, 40, 55, 80, 85, 90, and 95°C.

Upper figure, with an addition of 20 mg/100 ml of AsA; lower figure, without an addition of AsA. 'Total catechins', the sum of −EC, −ECg, −EGC, and −EGCg. Initial concentration of 'total catechins' in green tea infusion with an addition of AsA, 93.3 mg/100 ml; in green tea infusion without an addition of AsA, 93.9 mg/100 ml. Symbols: ○, at 25°C; ●, at 40°C; △, at 55°C; ▲, at 80°C; □, at 85°C; ◪, at 90°C; ■, at 95°C.

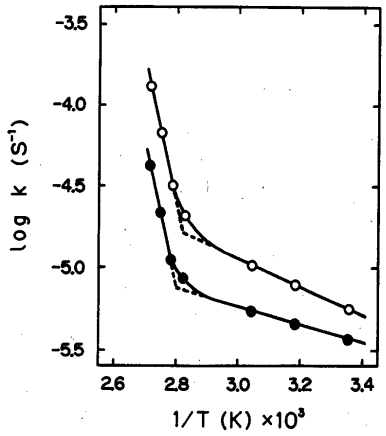

Fig. 6. Arrhenius Plot of Apparent First Order Reaction Rate Constant of 'Total Catechins' in Green Tea Infusions with or without an Addition of AsA.

Symbols: ●, in green tea infusion with 20 mg/100 ml of AsA; ○, in green tea infusion without an addition of AsA.

original infusion was 3.8 kcal/mol, while E_{a2} was 35.2 kcal/mol in the AsA-added and 38.4 kcal/mol in the original infusion. The turning point temperature on Arrhenius plot was also observed in both slightly acidic and original media at 82°C (Fig. 6). Therefore, a similar mode of reaction proceeded for isomerization of tea catechins in slightly acidic infusions.

In summary, isomerization reaction, a dominant change of −EC in thermal processing, was accelerated at pH higher than 6.0, and inhibited at pH lower than 5.0.

The reaction of tea catechins, −EC, −ECg, −EGC, and −EGCg in green tea infusions, fitted an apparent first order reaction kinetics at temperatures below 95°C. The rate constants measured from 25 to 95°C were dependent upon the kinds of catechins; the rate constant of −EGCg was the least and that of −ECg was the largest. The difference was 3 to 10 times depending on temperature.

A specific turning point temperature on Arrhenius plot was observed for four kinds of catechins at 82°C without exception. A great difference was observed between the activation energies measured below and above the turning point temperature. The activation energy at higher temperatures was 7.3 to 11.4 times as large as that at lower temperatures for four kinds of catechins.

A similar turning point temperature was observed in slightly acidic infusion by an addition of AsA, an inhibitant to isomerization. It was attributed to the role of AsA as an acidulant, not a reductant.

Therefore, a simple time–temperature relationship was not established in the thermal stability of tea catechins due to the existence of a turning point temperature on the Arrhenius plot. The stability of catechins in green tea infusions was subject to temperature rather than heating time.

We were additionally informed that a careful consideration of time–temperature infusing conditions was required to extract natural catechins from green tea for quality evaluation or preparation of ingredients for pathological studies.

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